LONG RANGE GENE ENHANCERS REGULATE C-MYC RNA THROUGH CHROMATIN LOOPING IN PROSTATE CANCER

by
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ABSTRACT

It has been previously shown in a semi-quanitative way that c-Myc confers androgen-independent prostate cancer cell growth, and a significant finding from this work showed AR regulates c-Myc at a posttranscriptional level. The data presented here shows that c-Myc protein expression is required for both normal and prostate cell growth, however c-Myc mRNA and protein is expressed and maintained differently between these two types of cell lines.

Using a new method, SmartFlares, we are able to show from a single cell method without having to extract RNA that c-Myc mRNA remains constant when treated with bicalutamide, while c-Myc protein decreases. We propose that an enhancer 21.7 kb upstream of the c-Myc gene becomes activated and loops to the c-Myc promoter in prostate cancer.

Advisor: John T. Isaacs, PhD

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INTRODUCTION

In the normal prostate, intermediate cells that express the androgen receptor (AR) migrate from the basal to the luminal layer and terminally differentiate into AR and prostate specific antigen (PSA) positive secretory-luminal cells. AR is a nuclear receptor and its main role is to function as a DNA-binding transcription factor. It is also thought to play a role in the cell cycle as a DNA licensing factor. Studies have shown that in the human prostate a small fraction of proliferating cells in the basal compartment do not express AR protein, while the AR positive luminal cells are proliferative quiescent. When AR is knocked out selectively in luminal cells, these cells become hyper proliferative and do not terminally differentiate.[1] In prostate cancer, AR no longer serves this repressive function, and is thought to promote DNA replication pushing the cell cycle forward through a gain of function in c-Myc regulation.[1-6] The androgen receptor was shown to promote ligand-independent progression of prostate cancer by upregulating c-Myc.[2-6] However, it is not the AR that is acting on the normal androgen-dependent gene expression program, but instead a distinct program resulting in androgen-independent growth.[7]

It has been previously shown in a semi-quanitative way that c-Myc confers androgen-independent prostate cancer cell growth, and a significant finding from this work showed AR regulates c-Myc at a posttranscriptional level.[8] The data presented here shows that c-Myc protein expression is required for both normal and prostate cell growth, however c-Myc mRNA and protein is expressed and maintained differently between these two types of cell lines.

Using an immortalized human prostate epithelial cell line, 957 E/hTert, we show that c-Myc expression is necessary for cellular growth. Knockdown β-catenin or TCF-4 causes growth suppression due to simultaneous loss of c-Myc expression. These cells do not express androgen receptor (AR), and when transfected by AR maintain a growth suppressive response to R1881 (synthetic androgen) due to loss of c-Myc expression at both mRNA and protein levels. AR serves a repressive function in normal prostate cells causing them to become growth arrested in the presence of androgen. To overcome this, these cells downregulate AR in the presence of R1881 and overcome the growth arrested state.

Unlike this epithelial cell line, we show that prostate cancer cell lines (LNCaP, LAPC4, and CWR-22R_v1) respond differently. c-Myc expression is no longer suppressed by TCF-4 knockdown or the presence of R1881. Treating these prostate cancer cell lines with the anti-androgen, bicalutamide, causes loss of c-Myc expression at the protein level and suppresses cell growth. Using a new method, SmartFlares, developed by EMD Millipore we are able to show from a single cell method without having to extract RNA that c-Myc mRNA remains constant (AR mRNA is upregulated) when treated with bicalutamide. AR no longer serves a repressive function in prostate cancer cells, and instead promotes the cell cycle. Loss of AR protein leads to a loss of c-Myc protein expression. We propose that an enhancer 21.7 kb upstream of the c-Myc gene becomes activated and loops to the promoter in prostate cancer maintaining c-Myc mRNA.

METHODS

Bicalutamide Treatment Experiments Methods

Cell Culture

LAPC4 cells were maintained in culture in a humidified 5% CO2 atmosphere at 37°C in complete medium consisting of IMDM supplemented with 10% fetal bovine serum, and 1 nM R1881.

LNCaP cells were maintained in culture in a humidified 5% CO2 atmosphere at 37°C in complete medium consisting of RPMI supplemented with 10% fetal bovine serum.

Plating of Cells

For RT-PCR

LAPC4 and LNCaP cell lines were plated at a density of one million cells per T75 flask. The cells were allowed to sit overnight for attachment.

For SmartFlares

LAPC4 and LNCaP cell lines were plated at a density of one million cells per T25 flask. The cells were allowed to sit overnight for attachment.

Bicalutamide Treatment

Following overnight incubation, the media was exchanged for media supplemented with 10 μM bicalutamide. Control culture media was replaced with media supplemented with an equivalent volume of 100% DMSO.

Harvesting Cells

For RT-PCR

Harvesting of cells occurred 96 hours (LNCaP) and 120 hours (LAPC4) post-treatment with bicalutamide. Total number of cells were quantified using a Nexcelom Bioluminescence Cellometer and trypan blue.

For SmartFlares

Harvesting of cells occurred 24 hours post-treatment with bicalutamide.

Total RNA Extraction

Total RNA was isolated from 2.5 million cells harvested from each flask using the RNeasy Mini Kit (Qiagen) according to the manufacturers' instructions.

cDNA Synthesis

Total RNA from each sample (5 μg) was reverse transcribed using the iScript™ cDNA synthesis kit (Bio-Rad) using their blend of random hexamers to anchored oligo-dT primers in a 20 μL reaction according to the manufacturer's protocol. No reverse transcriptase controls were prepared from untreated cells' total RNA and no template controls were prepared with water in place of total RNA to indicate potential genomic DNA contamination in isolated total RNA and contamination of reagents, respectively.

RNA Quality Determination

The concentration and purity of the total RNA was spectrometrically determined using a NanoDrop 1000™ (Thermo Scientific). The absorbance ratio A260/A280 was used as an indicator of protein contamination and A260/A230 as an indicator of polysaccharide, phenol, and/or chaotropic salt contamination.

Primers and real-time PCR (qPCR)

c-Myc gene expression was determined using real-time PCR (qPCR). Oligonucleotide primers were designed using IDT DNA software (www.idtdna.com). The c-Myc forward primer sequence selected was 5' - GTA GTG GAA AAC CAG CAG CC – 3' and the c-Myc reverse primer sequence selected was 5'- AGA AAT ACG GCT GCA CCG AG – 3'. GAPDH was used as a housekeeping control gene with a forward primer sequence of 5'- TCC AAA ATC AAG TGG GGC GA – 3' and a reverse primer sequence of 5'- AAA TGA GCC CCA GCC TTC TC – 3'. The 20 µL reactions contained 1 µL of undiluted cDNA or no-RT control, 1 µL (10 µM stock) of each primer, 7 µL nuclease free H2O, and 10 µL of the SsoFast™ EvaGreen® Supermix. qPCR run was performed on a CFX 96 (Bio-Rad) with the following cycle parameters: 95°C for 30 s, 40 cycles of 95°C for 5 s, 60°C for 5 s and 60°C for 5 s.

SmartFlare Probes

The probes received were resuspended dropwise in 50 µL of sterile, nuclease-free water according to the manufacturer's protocol (EMD Millipore). Each probe was vortexed for 10 seconds. The resuspeneded probes were stored at room temperature. The probes were diluted at a ratio of 1 to 20 (water:PBS) prior to use. 50 µL of the diluted, desired probe was added to the appropriate T25 flask. The cells were then incubated overnight in a humidified 5% CO2 atmosphere at 37°C. Four probes were used: cy5-scramble control, cy3-scramble control, cy5-Androgen Receptor (AR), cy3-c-Myc. The cy5 and cy3 scramble probes was used to measure background fluorescence while the other probes, cy5-AR and cy3-c-Myc were used for detection of AR and c-Myc respectively.

Flow cytometry

Post 16-hour incubation each T25 flask was harvested. Cells were resuspended in MACS buffer. The cells were then filtered into 5 mL polystyrene round-bottom tube with cell-strainer cap from Corning.

Flow cytometry was performed using a four color BD FACSCalibur.

Electrophoretic Mobility Shift Assay (EMSA)

EMSA was performed using a kit from Promega (Cat # E3050). This involves incubating a ^{32}P end labeled double stranded oligonucleotide probe (i.e. 5'gctattgctgttctaattacctcattgtct3') containing the putative ARE (underlined) from the 3' c-Myc enhancer (Ref) with bacterial expressed and purified AR DNA binding domain in the gel shift binding buffer. Bound probe was separated from unbound probe by gel electrophoresis in 5% polyacrylamide at 120 V for 1.5 hrs. Gels were dried and exposed to X-ray film. The specificity of the DNA binding domain for the putative binding site is confirmed by competition assays using mutated oligonucleotides with mutations (denoted by capital letters) in the putative AR binding site (i.e. mutation 1: 5'gctattgctgttcCaattacctcattgtct3' and mutation 2: 5'gctattgctgtCcCaattacctcattgtct3') vs. a non-specific mutation outside of the ARE (i.e. mutation 3 5'gctattgctgttctaattacctcattgCcC3').

Methods for experiments not listed[1]

RESULTS

c-Myc Knockdown Growth Suppression

Normal prostate epithelial cells, hTert, LNCaP, LAPC4, CWR-22R_v1 and VCaP cells were treated with either scrambled sh-RNA or c-Myc sh-RNA (Figure 1). Knockdown of c-Myc, using the c-Myc sh-RNA, inhibited cell growth by at least 78% for each cell line when compared to controls.

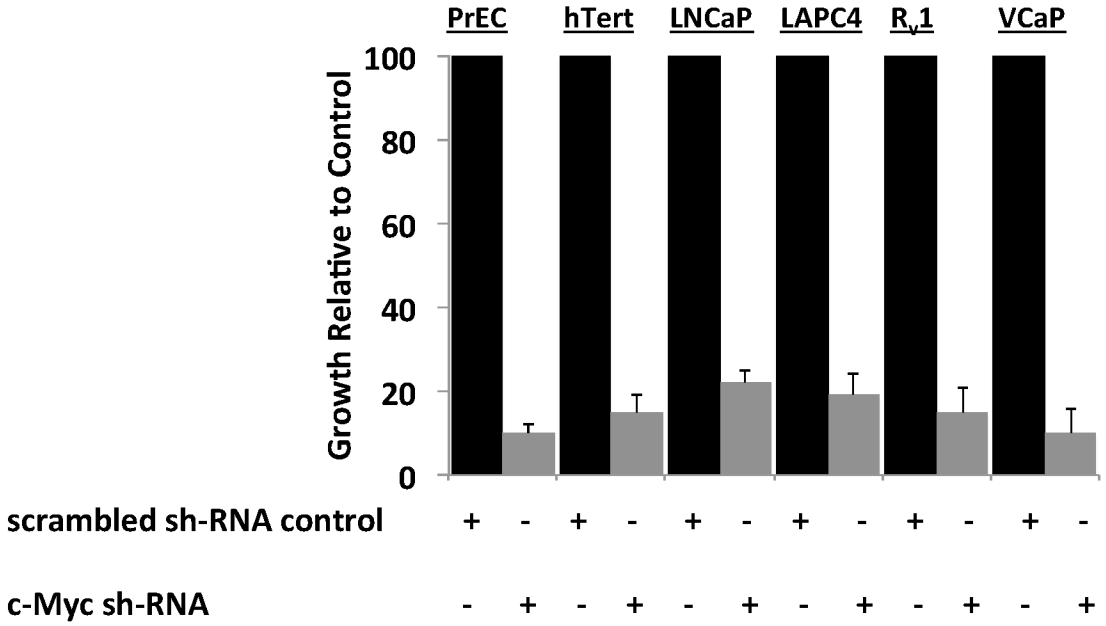

Figure 1: Knockdown of c-Myc mRNA through sh-RNA inhibits cell growth in normal (PrEC, 957E/hTert) and prostate cancer (LNCaP, LAPC4, CWR22R_V1, VCaP) cell lines.

Expression of β-catenin, TCF-4, LEF1, and c-Myc: Normal versus Prostate Cancer

Normal (957E/hTert, PrEC, and PrEC hTert) and Prostate Cancer (LNCaP, LAPC4, CWR-22R_v1 and VCaP) cell lines were evaluated for their protein expression of β-catenin, TCF-4, LEF1, and c-Myc using cytoplasmic and nuclear extracts (Figure 2). In all of the cell lines, β-catenin was expressed higher in the cytoplasmic fractions. The normal prostate cells expressed TCF-4 in both fractions (higher level in nuclear). The

prostate cancer cells show loss of TCF-4 expression. LNCaP cells show low expression in the nuclear fractions. CWR-22R_v1 cells have expression of TCF-4 in its nuclear fractions. LAPC4 cells have the highest expression of any cell line in its nuclear fraction, and have low expression in its cytoplasmic fractions. VCaP cells do not express TCF-4. LEF1 expression is absent from all of the normal prostate cell lines. The prostate cancer cell lines all express LEF1 in both cytoplasmic and nuclear fractions except for LNCaP cells (nuclear fraction only). LEF1 was shown to be upregulated in androgen-independent prostate cancer cell lines.[9] Increases in LEF1 expression increased AR expression and consequently enhanced growth and invasion ability of cells. c-Myc expression was higher in prostate cancer cell lines. In all cell lines, expression was higher in the nuclear fractions. The androgen receptor was shown to promote ligand-independent progression of prostate cancer by upregulating c-Myc.[9] The increase seen in LEF1 expression correlates directly with c-Myc, as there is an increase in AR due to LEF1.

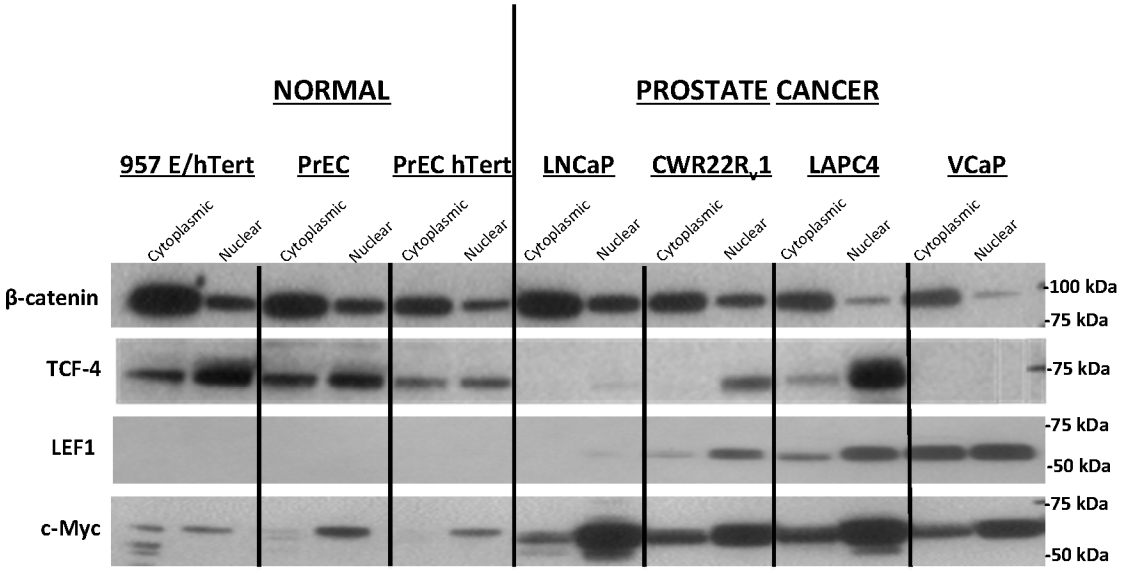

Figure 2: Comparison of the expression of β-catenin, TCF-4, LEF1, and c-Myc protein in normal and prostate cancer cell lines.

β-catenin and TCF-4 knockdown in 957 E/hTert cells

Knockdown of down β-catenin or TCF-4 leads to loss of 957 E/hTert cell growth (Figure 3A). Protein expression in Figure 3B and 3C show that knockdown of β-catenin or TCF-4 leads to a decrease of c-Myc expression after one day, followed by complete loss of expression. Loss of c-Myc expression through knockdown of β-catenin or TCF-4 would be expected to produce this inhibitory growth effect.

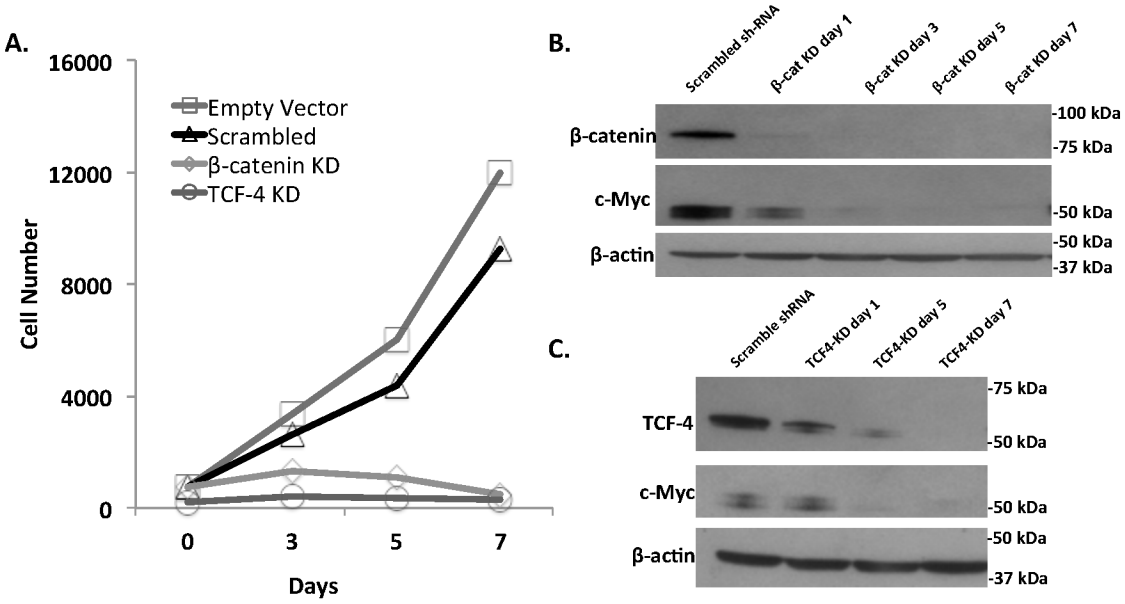

Figure 3: (A) Knockdown of β-catenin or TCF-4. (B) Protein expression of c-Myc in β-catenin knockdown cells. (C) Protein expression of c-Myc in TCF-4 knockdown cells.

Effect of treatment of 957 E/hTert and 957 E/hTert Lenti-AR cells with R1881

957 E/hTert and 957 E/hTert Lenti-AR cells growth was determined in the absence and presence of R1881 (Figure 4A). 957 E/hTert cells do not express AR (Figure 4A insert) and treatment with R1881 does not affect cell growth. 957 E/hTert cells transfected with lentiviral-AR have similar cell growth to 957 E/hTert cells in the absence of R1881, however their growth is suppressed in the presence of R1881.

Expression of AR inhibits cell growth in the presence of R1881 (synthetic androgen), showing the repressive function of AR in prostate cells.

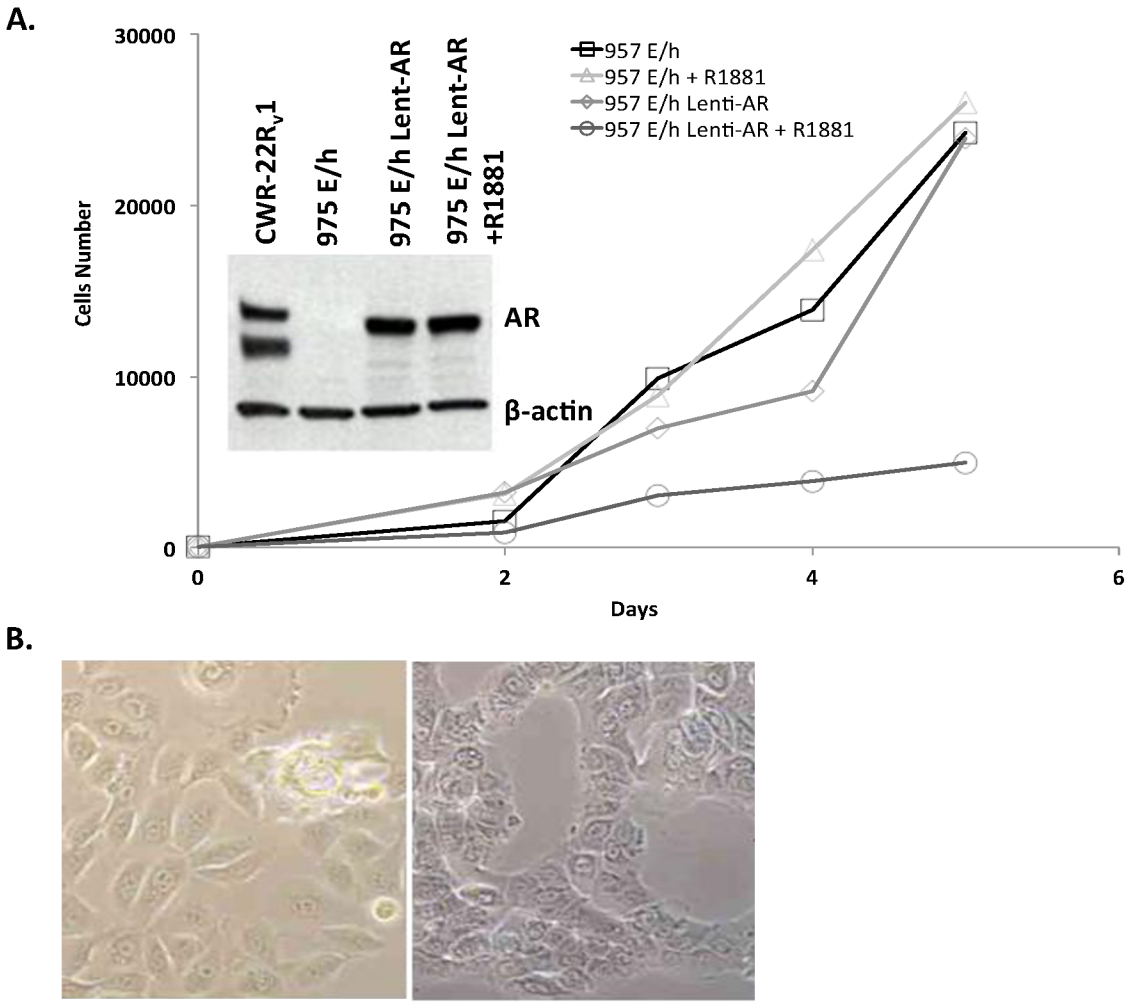

Figure 4: (A) 957E/hTert and 957E/hTert Lenti-AR cell growth +/- R1881. (Insert) AR protein expression compared to control CWR22R$_1$1 (B) Histology of 957E/hTert (left) compared to 957E/hTert Lenti-AR (right)

Effect of R1881 treatment on c-Myc mRNA and protein in 957 E/hTert and 957 E/hTert Lenti-AR cells

Previous studies documented that AR-induced growth suppression of prostate epithelial cells is associated with decreased expression of c-Myc.[2] Real-time PCR (RT-PCR) of these cells demonstrated that in the presence of R1881, c-Myc mRNA in 957 E/hTert cells was decreased more than 2 fold (Figure 5A). 957 E/hTert cells were

infected stably with c-Myc retrovirus and tested for their clonogenic ability to grow in the presence of R1881 (Figure 5B). 957 E/hTert Lenti-AR that expressed exogenous c-Myc were more clonogenic than 957 E/hTert Lenti-AR cells in the presence of R1881. In Figure 5C, the protein expression of c-Myc in 957 E/hTert and 957 E/hTert Lenti-AR cells was evaluated in cytoplasmic and nuclear fractions. c-Myc was expressed in the nuclear fractions. 957 E/hTert Lenti-AR lose some expression of c-Myc after 1 hour of being cultured in R1881, and all detectable expression is lost after 24 hours. Both were expected results that correlate with the growth curves seen in Figure 4. Treating the cells with MG-132 (proteasome inhibiter) in the presence of R1881 rescued c-Myc expression. Both cytoplasmic and nuclear fractions show the presence of c-Myc at higher levels. The presence of R1881 could lead to a signal for c-Myc to be targeted for ubiquination followed by proteasome degradation.

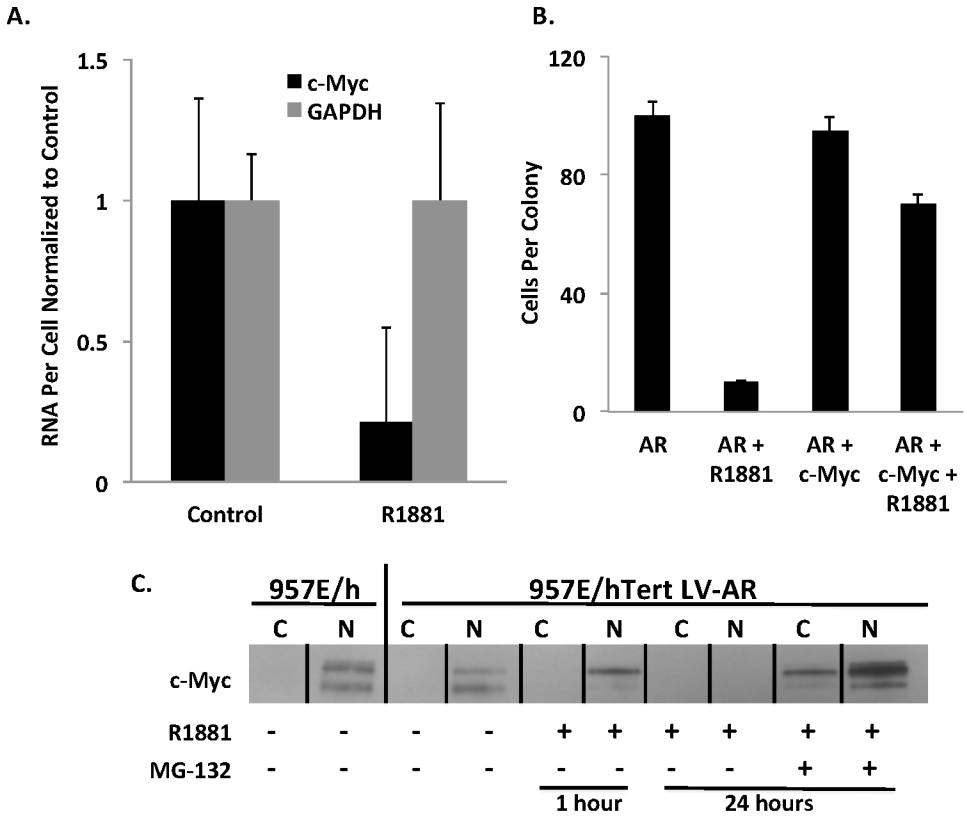

Figure 5: (A) 957E/hTert c-Myc RNA expression +/- R1881. (B) 957E/hTert cells were transfected with AR. Growth was suppressed with R1881 and rescued with exogenous c-Myc. (C) c-Myc protein expression +/- R1881 and +/- MG-132.

957 E/hTert Lenti-AR cells lose expression of AR in the presence of R1881

Using flow cytometry it can be demonstrated that there are two populations of AR expressing 957 E/hTert Lenti-AR cells (Figure 6A) In the untreated cells (top panel), the majority of cells expressed high AR (96.4%). Treatment of these cells with R1881 leads to a decrease in AR expression with the majority of the cells expressing low AR (96.2%). Through Western Blot analysis, R1881 treatment for as little as two days with R1881 reduces AR protein expression, and after 24 days it becomes undetectable. Removal of R1881 treatment does not recover AR expression even after 63 days seen in Figure 6B. Growth suppression of high AR expressing occurs in the presence of R1881. In Figure 6C, it can be seen that cells with low AR in the presence or absence of R1881 show the same growth potential as high AR expressing cells. Treatment of high expressing AR cells leads to growth suppression. To see if other cell lines behave similarly, in Figure 6D, PC3 and DU145 cells were also transfected with AR. Expression of AR and c-Myc was tested in the absence and presence of R1881. R1881 stabilizes expression of AR in PC3 AR and DU145 AR cells, but does not in 957 E/hTert Lenti-AR cells. c-Myc expression is also constant in PC3 AR and DU145 cells in the absence and presence of R1881. c-Myc expression is lost in 957 E/hTert Lenti-AR cells in the presence of R1881.

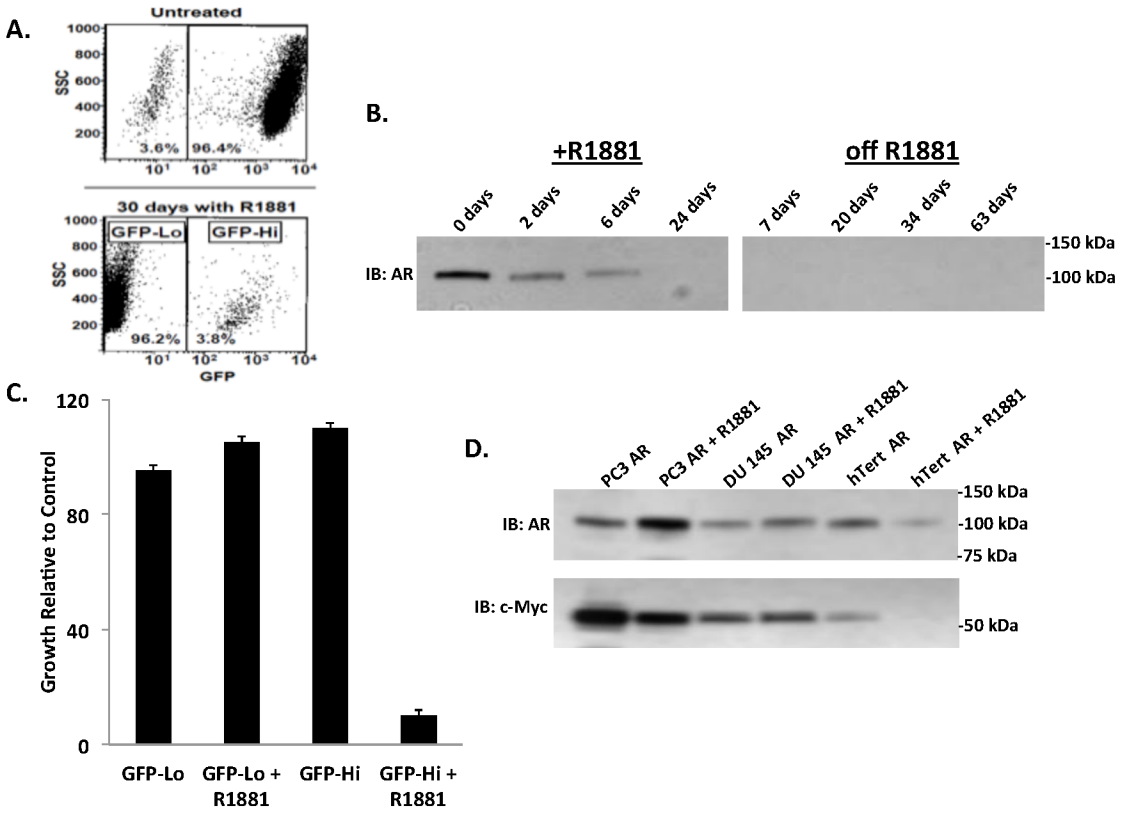

Figure 6: (A) Flow cytometry of 957E/hTert Lenti-AR +/- R1881. (B) Protein expression of AR during R1881 treatment and after removal of R1881. (C) Growth of the two populations of 957E/hTert Lenti-AR cells. (D) Expression of AR and c-Myc protein in PC3 and DU145 cells transfected with AR +/- R1881.

TCF-4 Knockdown Has No Effect On LNCaP Cell Growth or c-Myc Expression

Knockdown of TCF-4 does not affect c-Myc protein expression (Figure 7A and 7C).

TCF-4 knockdown LNCaP cells show similar cell growth to the control (Figure 7B).

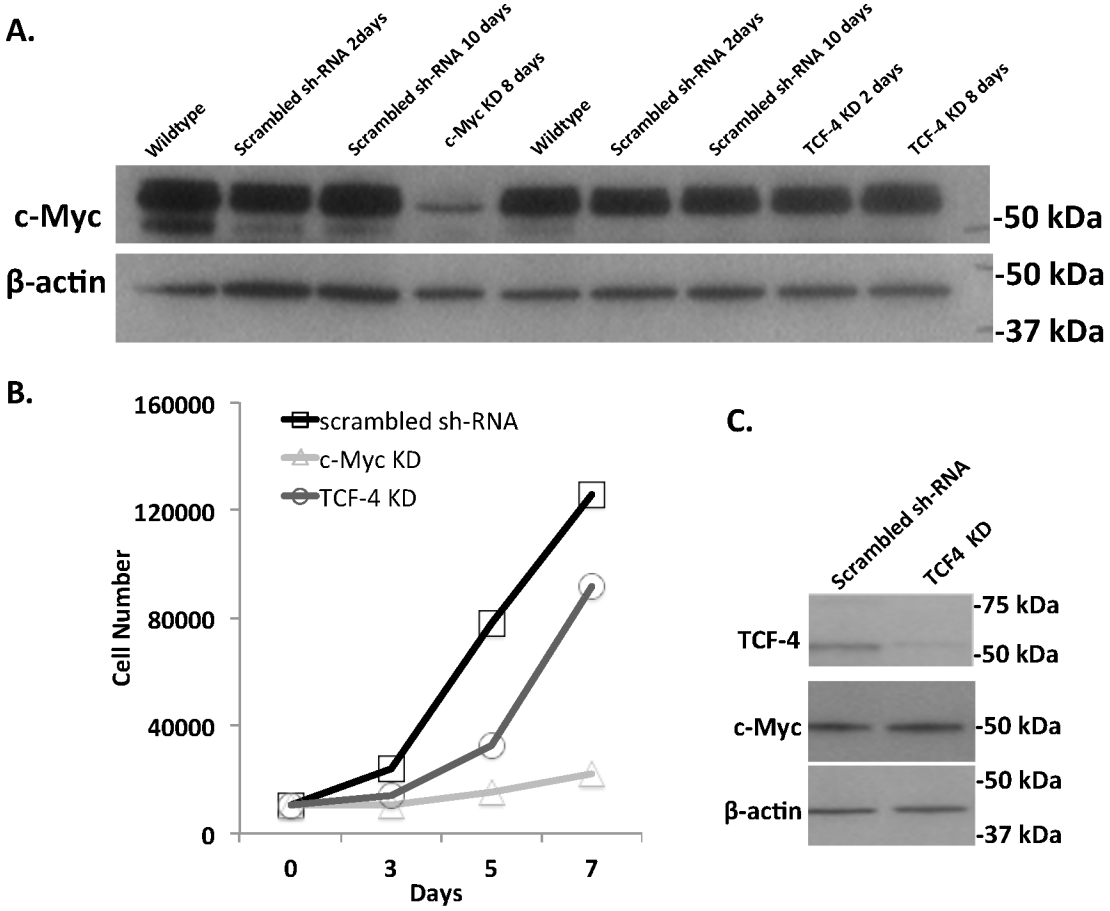

Figure 7: (A) c-Myc protein expression in LNCaP cells with c-Myc or TCF-4 knockdown. (B) LNCaP cell growth in c-Myc or TCF-4 knockdown. (C) c-Myc protein expression in LNCaP cells with TCF-4 knockdown.

<u>TCF-4 Knockdown Has No Effect On LAPC4 and CWR-22R_v1 Cell Growth or c-Myc expression</u>

TCF-4 knockdown was also done in CWR-22R_v1 and LAPC4 cells (Figure 8A). TCF-4 knockdown had no effect on c-Myc protein expression. The growth potential of both cells lines with the TCF-4 knockdown are similar to the respective controls (Figure 8B and Figure 8C).

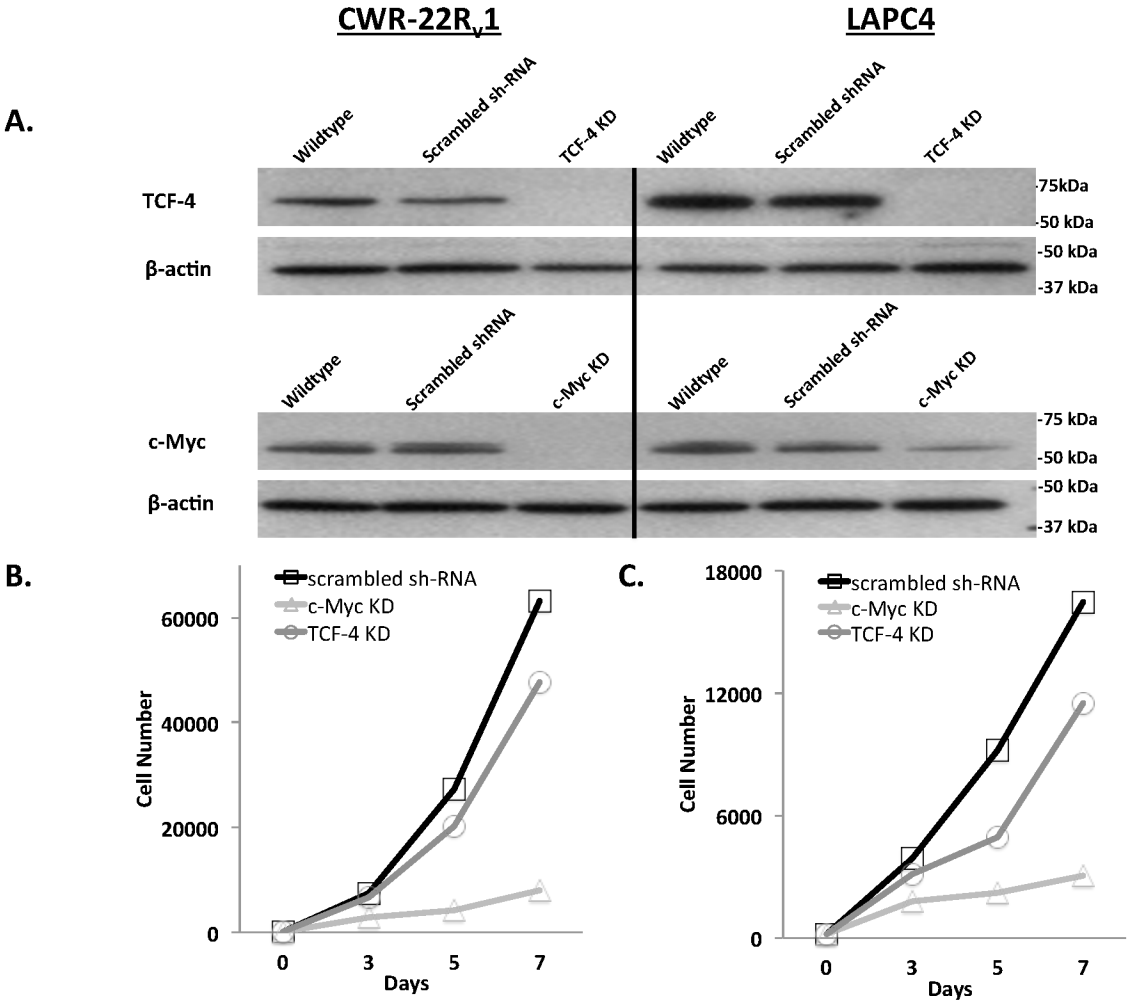

Figure 8: (A) TCF-4 or c-Myc knockdown in CWR-22R$_V$1 and LAPC4 cells. (B) c-Myc or TCF-4 knockdown in CWR-22R$_V$1. (C) c-Myc or TCF-4 knockdown in and LAPC4 cells.

Effect of 10 μM bicalutamide on c-Myc and other replication factors in LNCaP cells

10 μM bicalutamide arrested LNCaP cells had decreased c-Myc protein (Figure 9A). Other factors associated with the cell cycle, cell division cycle 6 (cdc6) protein and phosphorylation of retinoblastoma protein on serine 608 (Ph-Rb Ser608), were also shown to be decreased during treatment with 10 μM bicalutamide (Figure 9B). A time recovery was also shown for each of these proteins after release into R1881. After 48 hours all three proteins recovered to normal levels.

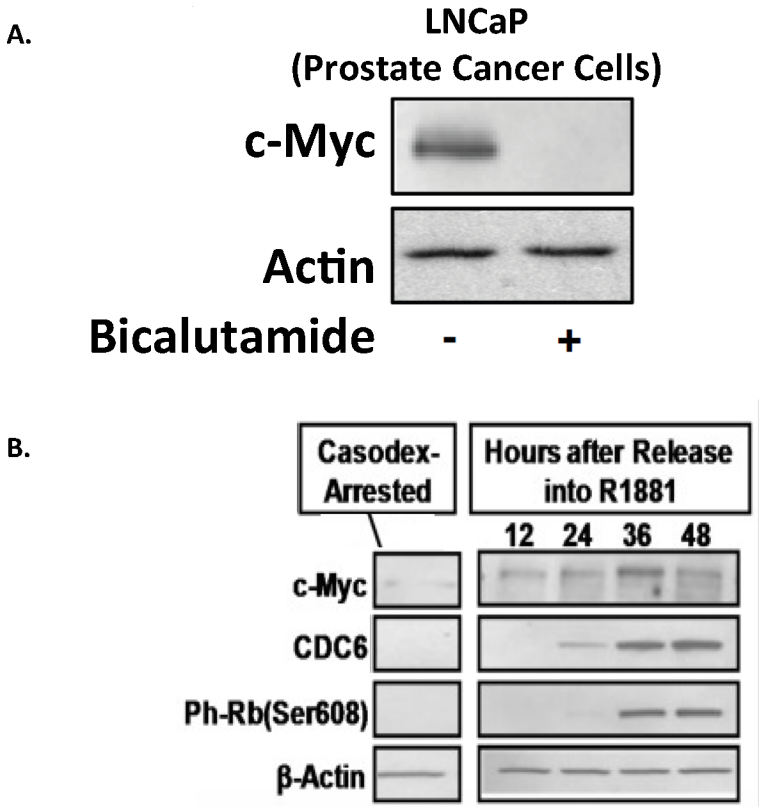

Figure 9: (A) Knockdown of c-Myc with 10 μM bicalutamide in LNCaP cells. (B) Recovery of c-Myc and other replication factors after treatment with 10 μM bicalutamide.

Expression of AR and c-Myc in LNCaP cells

Figure 10 shows images of LNCaP cell expression using markers for DNA (DAPI), c-Myc, and AR. The merge of all the images show that there are cells that are positive for AR (8/18) or AR and c-Myc (10/18). There are no cells that are positive for c-Myc and negative for AR.

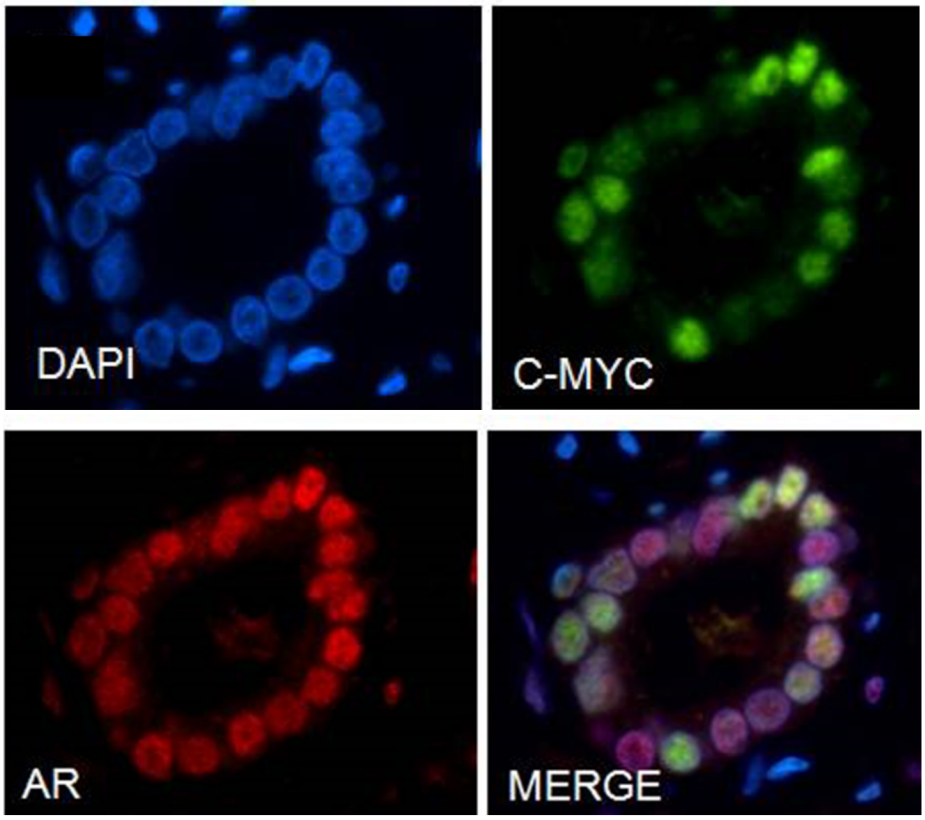

Figure 10: c-Myc and AR expression in LNCaP cells.

LNCaP and LAPC4 cells treated with bicalutamide undergo growth suppression without a reduction in c-Myc expression

Treatment of LNCaP and LAPC4 cells with 10 μM bicalutamide has a profound effect on cell growth. Both cell lines experienced a growth decrease by 54% and 38% respectively in Figure 11A. In 2003, Beach showed that c-Myc confers androgen-independent prostate cancer cell growth.[8] The Beach group showed using PCR that this cell growth suppression by bicalutamide is not caused by a transcriptional decrease in c-Myc, but by a decrease in levels of c-Myc protein. Using real-time PCR (RT-PCR), in Figure 11B, we were able to replicate that in the presence of bicalutamide, the c-Myc mRNA levels remained the same as controls. These results were in agreement with those seen by Beach.

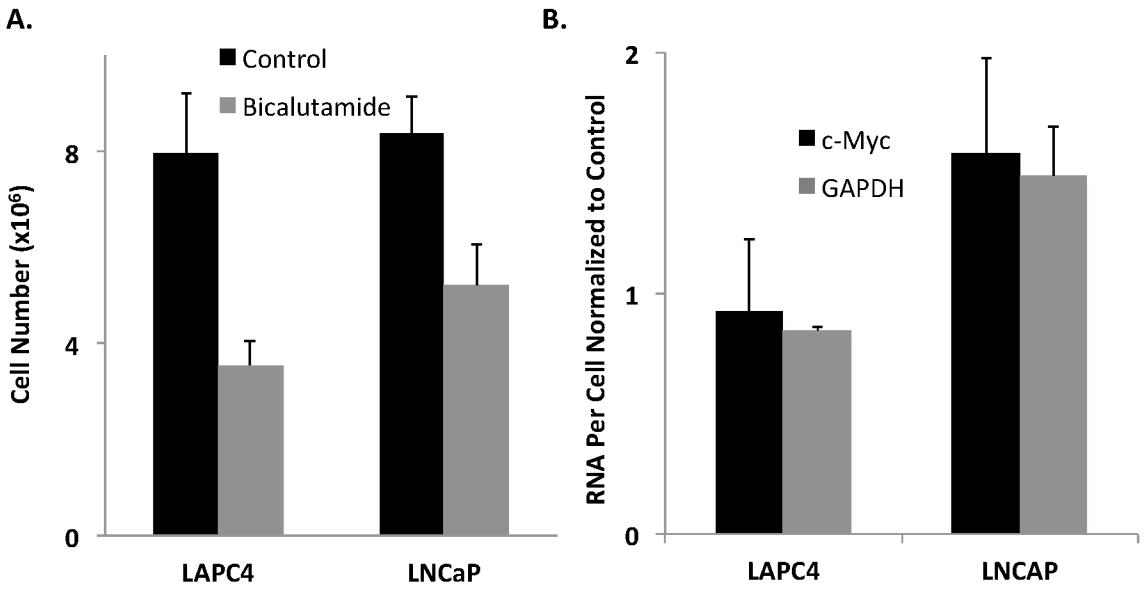

Figure 11: (A) Cell growth suppression of LAPC4 and LNCaP cells treated with 10 μM bicalutamide. (B) RNA expression of c-Myc in LAPC4 and LNCaP cells treated with 10 μM bicalutamide.

Single cell analysis of c-Myc and AR expression in LNCaP AND LAPC4 cells using SmartFlares

To approach the quantification of RNA levels of c-Myc from a single cell method that did not require RNA isolation, we used a new technology, SmartFlares: a set of RNA detection probes developed by EMD Millipore and are endocytosed by live cells.[10,11] There is no need for transfection and/or RNA amplification or detection. Each SmartFlare consists of three components – a gold nanoparticle, oligonucleotide cDNA sequence complementary to the target RNA, and a fluorophore. The gold nanoparticle is coated with multiple copies of the complementary oligonucleotide sequence (reporter strand). The reporter strands each bear a fluorophore that is quenched by the gold nanoparticle. When the target RNA (capture strand) binds the reporter strand, the fluorophore is displaced and can be detected.

Cells were plated and allowed to attach overnight before being treating with the desired SmartFlare for 16 hours. The cy5 and cy3 scramble probes were used to determine background fluorescence in order to distinguish a real signal. The regions gated as M1 in Figure 12A and B represent the region in which the background signal appears, and the region gated by M2 represents real c-Myc or AR signal respectively from the fluorophores released by the SmartFlares. The top two panels of Figure 12A represent c-Myc expression in untreated control LNCaP and LAPC4 cells resepectively, while the bottom panels represent the bicalutamide treated LNCaP and LAPC4 cells resepectively. The c-Myc signal is unchanged when comparing the control cells to the bicalutamide treated cells in both cell lines. The median c-Myc peak for the LNCaP control cells was 18.27, while the bicalutamide treated cells was 24.36. The median c-Myc peak for the LAPC4 control cells was 30.23, while the bicalutamide treated cells was 29.96. This was the expected result based upon the RT-PCR data. In order to design a control to test the ability of the SmartFlares to detect a change in expression, we used the Androgen Receptor (AR). The top panels in Figure 12B represent AR expression in untreated control LAPC4 and LNCaP cells resepectively, while the bottom panels represent the bicalutamide treated LNCaP and LAPC4 cells resepectively. In 2011, Brown showed that AR is reactivated in castrate resistant prostate cancer (CRPC) and leads to increased AR gene expression.[7] Cell lines treated with bicalutamide showed increased expression of AR. We show that bicalutamide treated cells have an increased expression in AR through an increase in median peak in both cell lines. The median AR peak for the LNCaP control cells was 922, while the bicalutamide treated cells was 1064. The median AR peak for the LAPC4 control cells was 1207, while the bicalutamide

treated cells was 1485. AR served as a positive control for the SmartFlare technology, showing that a signal change could be detected in both cell lines.

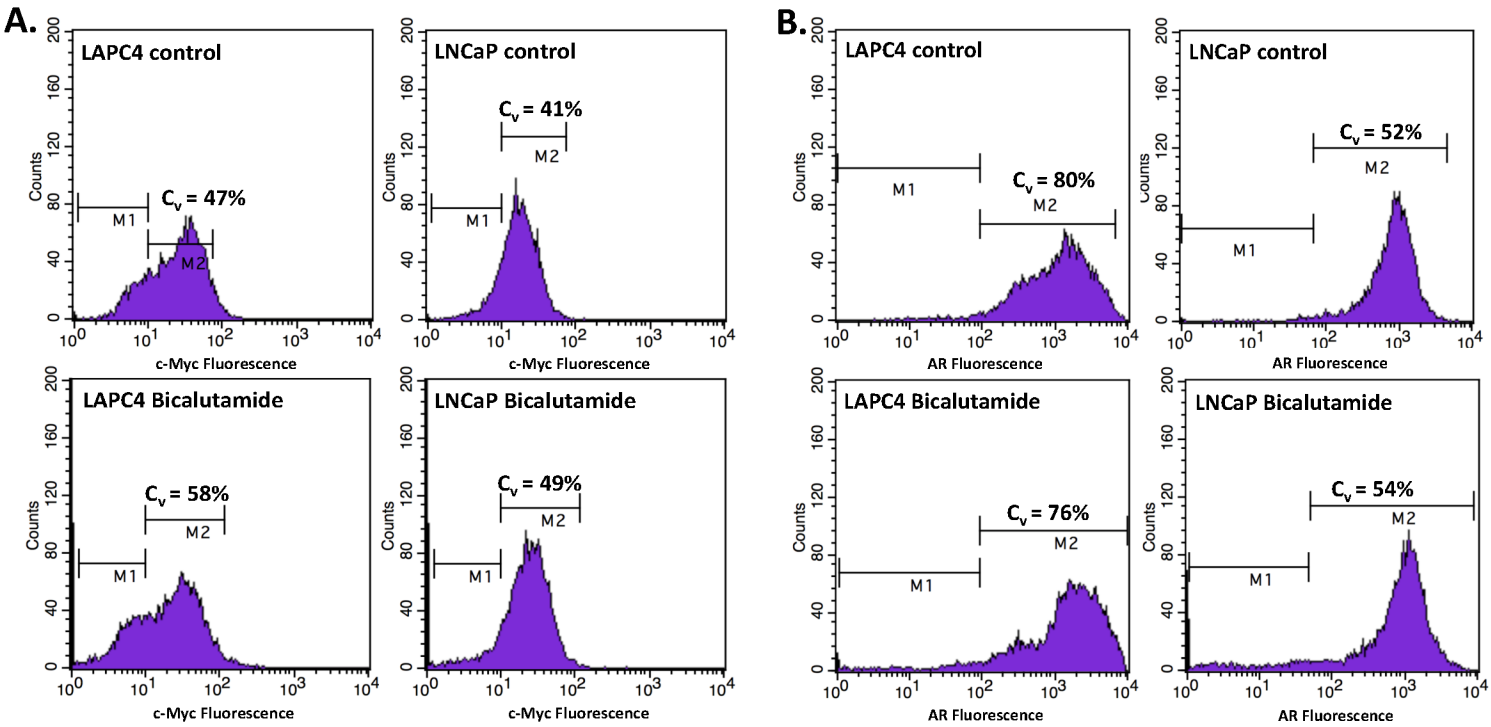

Figure 12: (A) c-Myc SmartFlare fluorescence +/- 10 µM bicalutamide in LAPC4 and LNCaP cells. (B) AR SmartFlare fluorescence +/- 10 µM bicalutamide in LAPC4 and LNCaP cells.

DISCUSSION

In normal tissue, c-Myc expression is necessary for cellular growth. This expression is mediated through factors β-catenin or TCF-4. Loss of either of these reduces or eliminates expression of c-Myc and causes growth suppression. Using prostate cancer cell lines (LNCaP, LAPC4, and CWR-22R_v1) we show that c-Myc expression is no longer suppressed by knockdown of β-catenin or TCF-4. However, c-Myc expression is still required for these cancerous cell lines to proliferate, and the RNA and/or protein expression is increased leading to hyper-proliferation

Bicalutamide, an anti androgen, suppresses the growth of prostate cancer cell lines (LNCaP, LAPC4) through knockdown of c-Myc protein, not c-Myc mRNA. Using RT-PCR we were able to show that c-Myc mRNA was not affected using bicalutamide. To show that this process occurred on an individual cell level, we used SmartFlares. Using this method, we are able to show that c-Myc RNA is unaffected when AR protein is knocked down through bicalutamide. AR protein plays an important role in preventing the degradation of c-Myc protein.

Regulation of c-Myc is complex and context specific

c-Myc regulation, has been shown to be tightly controlled, and can occur at several levels - transcriptional, transcript processing, and/or translational/post-translational. It has been shown that c-Myc can be regulated at both the mRNA and protein level. c-Myc mRNA and protein regulation was shown through a histone demethylase, JMJD1A.[12] Qi et al. showed JMJD1A to be essential for prostate cancer cell growth and survival by promoting recruitment of the androgen receptor (AR) to the c-Myc gene. This recruitment increased AR dependent transcription of c-Myc mRNA. It

was also shown to reduce the degradation of c-Myc protein levels by interacting with an E3 ubiquitin ligase and competing for the c-Myc binding site.[12]

SIRT3 and CIP2A are two modes of regulating c-Myc protein expression.[13,14] Gao et al. showed that downregulation of SIRT3 (sirtuin family) has been shown to increase c-Myc protein levels by decreasing its phosphorylation. Decreased levels of SIRT3 lead to suppression of c-Myc phosphorylation and promoted prostate cancer cell growth in vitro and in vivo.[13] Su et al. focused on CIP2A (cancerous inhibitor of protein phosphatase 2A). This oncoprotein was shown to be upregulated in prostate cancer. Upregulation of CIP2A was linked to increased protein levels of c-Myc.[14] This interaction could potentially lead to a target for therapeutics to regulate c-Myc protein levels.

It was also shown that c-Myc levels could be altered through upregulation of other factors. Loda et al. showed USP2a (Ubiquitin-specific protease 2a) to be upregulated in half of all prostate cancers. This protease was shown to downregulate microRNAs and lead to increased c-Myc expression.[15]

There is a wide range of diversity in the factors that regulate c-Myc. It is also important to point out that this regulation of c-Myc is a context and cell dependent process. Niu et al. shows that in ER-, PR-, AR+ breast cancer, AR expression upregulates let-7a mRNA, which decreases c-Myc levels.[16] The specificity of context adds another fold of complexity to the regulation of c-Myc. The evidence presented through these studies shows that as normal cells progress to cancer, normal mechanisms for regulation change allowing for cell survival and proliferation in prostate cancer.

Potential mechanism for c-Myc upregulation in prostate cancer

Yochum et al. described a model for a chromatin interaction network at the MYC gene locus in colorectal cancer. They describe the c-Myc proximal promoter serving as a "landing pad" for distal elements (response elements, enhancers, super-enhancers) coordinating chromatin structure. This network created has a proposed structure of loops that interact with the "landing pad" to bring all the elements involved into close proximity.[17,18]

Gray et al. proposed a new class of RNAs, enhancer RNAs (eRNAs) that facilitate enhancer-promoter interaction and/or activate promoter-driven transcription. Knockdown of these RNAs caused decreased expression of genes. The group also showed that eRNAs promote chromatin loops, keeping them close to the target promoter and preventing nonspecific activation of other genes.[19] Croce et al. and Rinn et al. showed that some of these loops attach to the MYC promoter through long noncoding RNAs (lncRNAs). Furthermore, specific lncRNAs are only expressed in cancerous tissue.[18,20] This finding provides evidence of certain regulatory elements becoming active only after the onset of cancer through upregulation of these lncRNAs.

Using the idea of chromatin loops, we believe that there is a 5' c-Myc enhancer located 21.7 kb away from the c-Myc promoter. This promoter is not active in normal prostate cells. Activation of this enhancer in cancer can lead to upregulation of c-Myc mRNA and increased proliferation, tumorigenicity, and survival. In Figure 13, we show the location of the upstream enhancer with proposed androgen receptor binding site.

Further studies need to be conducted using chromosome conformation capture (3C) analysis.

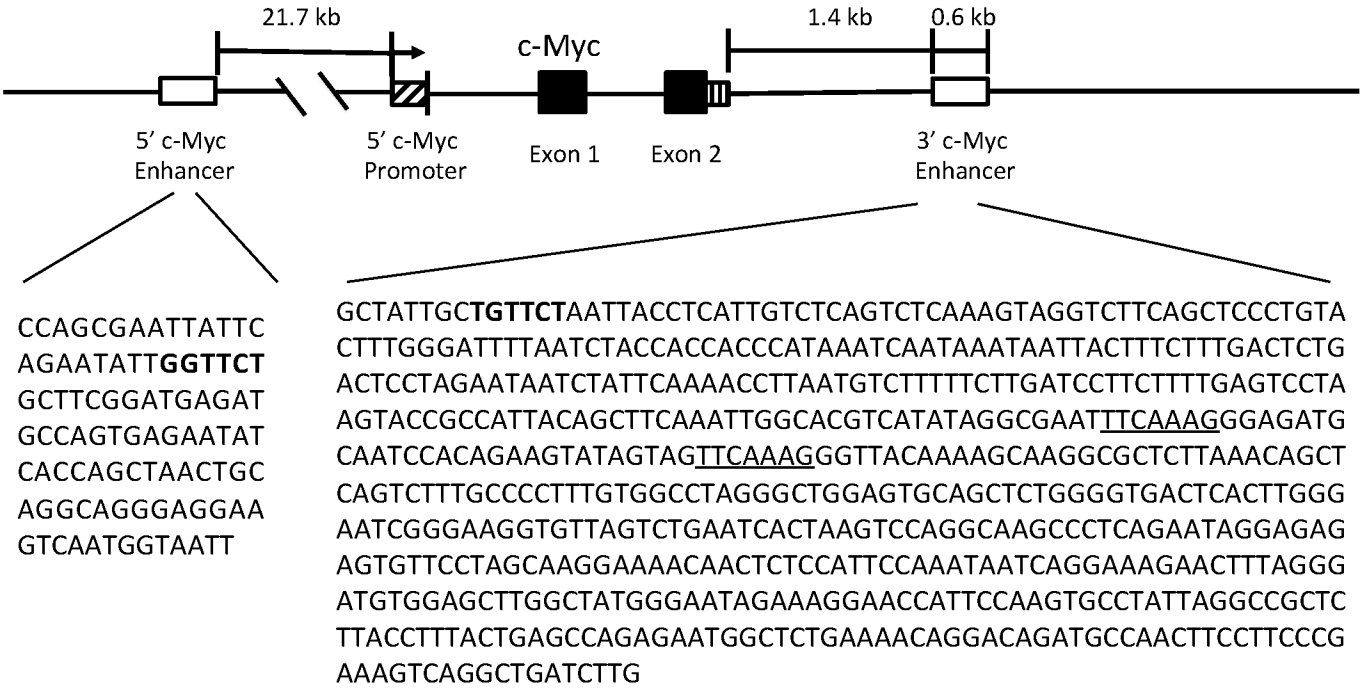

Figure 13: Proposed location of 5' c-Myc enhancer with androgen receptor binding site.

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RESEARCH/WORK EXPERIENCE:

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- Studied the role of the androgen receptor in the progression of castrate resistant prostate cancer. Under normal conditions, the androgen receptor does not act as a licensing factor at the ORC (origin of replication) during DNA replication, however during castrate resistant prostate cancer it drives the progression of the cell cycle.
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Spectrix Analytical Services / Bristol-Myers Squibb July 2012 – August 2013
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PUBLICATIONS

- **A. Kizewski**, M. A. Ilies, "Efficient and synergetic DNA delivery with pyridinium amphiphiles–gold nanoparticle composite systems having different packing parameters" Chem. Commun., 2016, 52, 60-63
- M. Ilies, T.V. Sommers, L.C. He, **A. Kizewski**, and V.D. Sharma "Pyridinium Amphiphiles in Gene Delivery – Present and Perspectives" In Amphiphiles: Molecular Assembly and Applications; Nagarajan, R.; ACS Symposium Series, ACS: Washington, DC, 2011, 23-38.

PRESENTATIONS

- **A. Kizewski**, D. Bartee, C.L. Freel Meyers, "Added Grease: Imine Pro-drug model for DXP synthase inhibitor MAP" Rotation Talks, The Johns Hopkins School of Medicine, Baltimore, MD, April 10, 2015
- **A. Kizewski**, M. Swierczewska, S. Lee, M.G. Pomper, "Non-cationic polymer nano-platform for gene delivery" Rotation Talks, The Johns Hopkins School of Medicine, Baltimore, MD, December 17, 2013
- **A. Kizewski**, M. A. Ilies, "Gold Nanoparticles Mediated Gene Delivery via Surface Charge Reversal with Pyridinium Amphiphiles", 15th American Society of Gene & Cell Therapy Annual Meeting, Philadelphia, PA, May 16-19, 2012.
- **A. Kizewski**, M. A. Ilies, "Gold Nanoparticles Mediated Gene Delivery via Surface Charge Reversal with Pyridinium Amphiphiles" TU School of Pharmacy Research Day, February 24, 2012
- **A. Kizewski**, M. A. Ilies, "Functionalized Gold Nanoparticles for Endocytosis Studies" TU School of Pharmacy Research Day, February 25, 2011 (2nd Prize in Undergraduate Research Section)